Formulation And Evaluation Of Floating Microspheres Of Cefdinir

Jeslin.D*[1], Suresh Kumar.P[2], Nithya Kalyani.K[1], Padmaja.V[1]

[1] Department of Pharmaceutics, Sree Balaji Medical College and Hospital Campus, Bharath Institute of Higher Education and Research, Chennai,Tamil Nadu, India

[2] Department of Pharmaceutics, The Erode College of Pharmacy, Erode ,Tamil Nadu, India

Corresponding Author:
Mrs.D.Jeslin,
Assistant professor,
Department of Pharmaceutics
Sree Balaji Medical College and Hospital Campus
Bharath Institute of Higher Education and Research
Chennai,Tamil Nadu, India
E.Mail: jeslin.123@gmail.com
Mobile: 9488366374

ABSTRACT

Various approaches have been used to retain the dosage form in the stomach as a way ofincreasing the gastric residence time (GRT), including floatation systems; high-density systems;

mucoadhesive systems; magnetic systems; unfoldable, extendible, or swellable systems; andsuperporous hydrogel systems. The aim of this study was to prepare and evaluate floatingmicrospheres of cefdinir for the prolongation of gastric residence time. Themicrospheres were prepared byCapillary Extrusion method.A full factorial design was applied to optimize the formulation. The optimum batch of microsphere exhibited smooth surfaces with good flow and packing properties, prolonged sustained drug release, remained buoyant for more than 12 hrs, high entrapment efficiency upto68%.Scanning electron microscopy confirmed the hollow structure with particle size in the order of190 μm. The studies revealed that increase in concentration of gum Karaya increased the drug release from the floating microspheres.

Key words: Cefdinir,Microspheres, Gum Karaya, factorial design, *in-vitro*, Buoyancy

INTRODUCTION

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site havemade an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems.1-3 They have varied applications and are prepared using assorted polymers.4 However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drugdelivery system with the absorbing membranes.5-8 This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres.Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimatecontact with the mucus layer, and specific targeting of drugs to the absorption site.9-12 .Gum karaya was selected as a polymer in thepreparation of mucoadhesive microspheres because of itsgood mucoadhesive and biodegradable properties.

Cefdinir is an expanded-spectrum, oral, third-generation cephlosporinantimicrobial agent active against Gram-positive and Gram-negative bacteria (PoojaMathuret al.,2010; Desai et al.,1993). It is used in the treatment of acute chronic bronchitis, rhinosinusitis, and pharyngitis and uncomplicated skin and skin-structure infections in adults and adolescents; it is indicated for acute otitis media, acute sinusitis, and community-acquired pneumonia (Wilson et al., 2001;Rubinstein et al.,1988).Cefdinir requires controlled release because of its short biological half-life of ~1.5 h(SH. Shahaet al.,2009).

MATERIALS AND METHODS

Cefdinir was a gift sample from (Aurobindo Pharmaceuticals Limited, Hyderabad, India). Sodium Tripoly Phosphatewere obtained from Hetro Pharmaceuticals, Hyderabad, India). Gum Karaya obtained from local market. Acetic acid was procured from Loba chemie Private Ltd. All other chemicals and reagents were analytical grade and used as received.

Experimental Design

A two factor three level full factorial design was used for systemic study of combination of drug and polymers. The linear interactive model is shown in following equation.

$$Y= b_0+b_1X_1+b_2X_2+b_3X_3+b_4X_1X_2+b_5X_1X_3+b_6X_2X_3$$

Where Y is the dependent variable, b_0 is the arithmetic mean response and b_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor from its low to high values. The interaction term (X_1X_2) shows how the response values change when two factors are simultaneously changed.

A 2^3 factorial design was applied for the experiment where two variables (X_1, X_2) were the amount matrix forming polymer and diluents. The levels of drug and polymer initially adjusted at 1:2 (-1+1) ratio for all ingredients such as Gum Karaya, Sodium Tripoly Phosphate, Acetic Acid. Further the quantity of drug and controlled release matrix forming polymer adjusted in combination as like 1:(1.5:0.5) i.e. (-1:0:+1). By this way totally 16 formulations were prepared, using Gum Karaya in different concentration by Capillary Extrusion method. The levels of polymer and crosslinking agent are set to low and high values are shown in the Table 1.

Table 1. Levels of Matrix forming polymer and diluents

Level	Gum Karaya in % (X_1)	Sodium Tripoly Phosphate in % (X_2)	Acetic Acid in % (X_3)
Low (-1)	1	1	1
High (+1)	1.5	3	2

Formulation of floating microspheres of Cefdinir

Gum Karayamicroparticles containing Cefdinir were prepared by a Capillary Extrusion method. The drug was dispersed in a stirred solution of Gum Karaya in 2% v/v Acetic acid until a uniform dispersion was obtained. The microparticles were formed by dropping the bubble free dispersion through a disposable syringe (with a nozzle of 1 mm inner diameter) into 20ml of gently agitated solution of the cross linking agent Sodium Tripoly Phosphate. The dropping rate was 30beads/minute. The falling distance was 5cm. The gelled microparticles were separated, after a reaction time of 2 hr, washed with deionised water and then air dried for 48 hours.Formulation are showed Table No.2.

Table 2. Formula for Floating Microsphere

S.No	Ingredients	Formulations					
		CFM1	CFM2	CFM3	CFM4	CFM5	CFM6
1	Cefdinir / Gum Karaya Ratio	1:1	1:1	1:1	1:1	1:1	1:1
2	Gum Karaya	1.5%	1.5%	1.5%	2%	2%	2%

3	Sodium Tripoly Phosphate	1%	2%	3%	1%	2%	3%
4	Acetic acid	1%	1.5%	2%	1%	1.5%	2%

Evaluation of Floating Microsphere

Determination of Drug Loading and Encapsulation Efficiency

About 50 mg of microparticles were digested in 100 ml of enzyme-free simulated gastric fluid (S.G.F: NaCl/HCl buffer; pH 1.2) and extracted completely during a period of 24 h. The solution was filtered and the amount of Cefdinir was measured spectrophotometrically (Shimadzu, Double-Beam Spectrophotometer 150-02, Japan) at 205 nm. Each determination was made in triplicate.

$$Drug\ Loading\ (\%) = \frac{Weight\ of\ the\ drug\ loaded\ in\ the\ microspheres}{Total\ weight\ of\ the\ microspheres} \times 100$$

$$Encapsulation\ Efficacy\ (\%) = \frac{Actual\ drug\ content\ in\ microspheres}{Theoretical\ drug\ content} \times 100$$

Determination of *In-vitro* buoyancy

Floating ability of microspheres was evaluated by Visual absorption method. For each formulation, 50 individual microspheres were placed in to 500ml of phosphate buffer pH 1.2 filled in types II (paddle) dissolution apparatus. Paddle rotation speed was at 100rpm, temperature was maintained at 37±0.5ºC. The number of floating microspheres was counted

visually after 24 hrs. Experiments were performed in triplicate and the percentage of floating microspheres was calculated according to the following equation.

$$Floating\ ability\ (\%) = \frac{Number\ of\ floating\ microspheres}{Total\ number\ of\ the\ microspheres} \times 100$$

Equilibrium swelling studies

A known weight (100 mg) of various Gum Karayamicroparticles without drug was placed in 500 ml of different solutions (distilled water, enzyme-free S.G.F. (HCl/NaCl solution; pH 1.2) and enzyme free S.I.F. (KH2PO4/NaOH buffer; pH 7.4) and allowed to swell for the required period of time at 37±0.5ºC using the USP dissolution apparatus with the dissolution basket assembly at 50 rpm. The microparticles were periodically removed, blotted with filter paper and their changes in weight were measured during the swelling until equilibrium was attained. Finally, the weight of the swollen microparticles was recorded after a time period of 4 h and the swelling ratio (SR) was then calculated from the

$$Equilibrium\ Swelling\ Studies\ (\%) = \frac{W_e - W_o}{W_o} \times 100$$

whereWo is the initial weight of the dry microparticles and We is the weight of the swollen microparticles at equilibrium swelling in the media. Each experiment was repeated three times and the average value±S.D. was taken.

***In vitro* release studies**

The release of Cefdinir from Gum Karayamicroparticles (equivalent to 25 mg of drug) was investigated using the USP dissolution paddle assembly with an agitation speed of 50 rpm in 250 ml of enzyme-free S.G.F. (HCl/NaCl solution containing 0.02% Tween 80, pH1.2) at 37± 0.5ºC. At appropriate time intervals, 5 ml samples were withdrawn and assayed

spectrophotometrically at 205 nm. The UV-absorption withmicroparticles without drug in dissolution testconditions was also measured. All dissolutionruns were performed in triplicate.

Scanning Electron Microscopy

The surface topography of the microparticles was examined using a scanning electron microscope Samples were coated with gold film under vacuum using a sputter coater and then investigated. Cross-sections were made in order to observe the core and internal structure of the microparticles.

RESULT AHD DISCUSSION

Based on the selected responses of two variables (X_1, X_2), the ratio of matrix forming polymers and (X_3) solvent were optimized and total 16 formulations were set to prepared. The compositions of drug, polymer and other ingredients of all formulations were presented in the Table 3.

Table 3. Factorial design of the formulation with results and constraints

Run No.	Variable			Response
	Gum Karaya in % (X_1)	Sodium Tripoly Phosphate in % (X_2)	Acetic Acid in % (X_3)	Drug loading efficiency in %
1	1	1	2	58.68
2	1	3	1	54.37
3	1.5	1	1	52.49

4	1	1	1	46.74
5	1	3	2	49.25
6	1.5	3	1	46.72
7	1.5	3	1	45.25
8	1	3	2	53.69
9	1.5	3	2	52.46
10	1.5	1	2	49.69
11	1.5	1	2	48.62
12	1	1	1	46.24
13	1	3	1	41.63
14	1.5	1	1	42.67
15	1	1	2	44.62
16	1.5	3	2	52.69

Evaluation of optimized formulations

The optimized formulations were identified based on constrains used in the experiment. The optimized formulations were made as microspheres to the method given in Table 3.and evaluated for efficiency of drug loading, results were presented in Table 3.

The optimized formulations were further studied for mechanism of drug release by fitting the in vitro drug release data into different kinetic models.

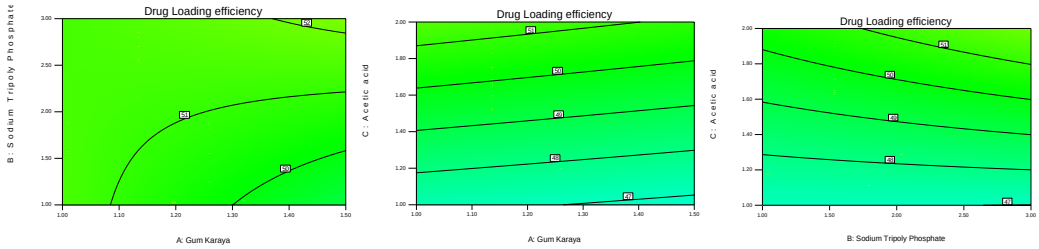


Figure 1: Contour plot showing the effect of various polymers concentration on efficiency of drug loading

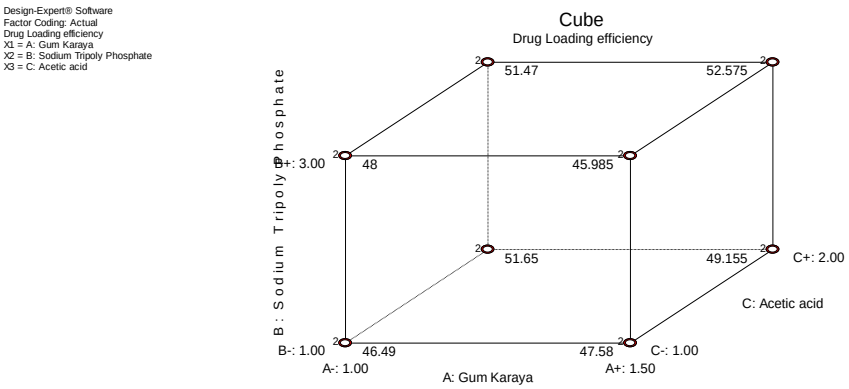
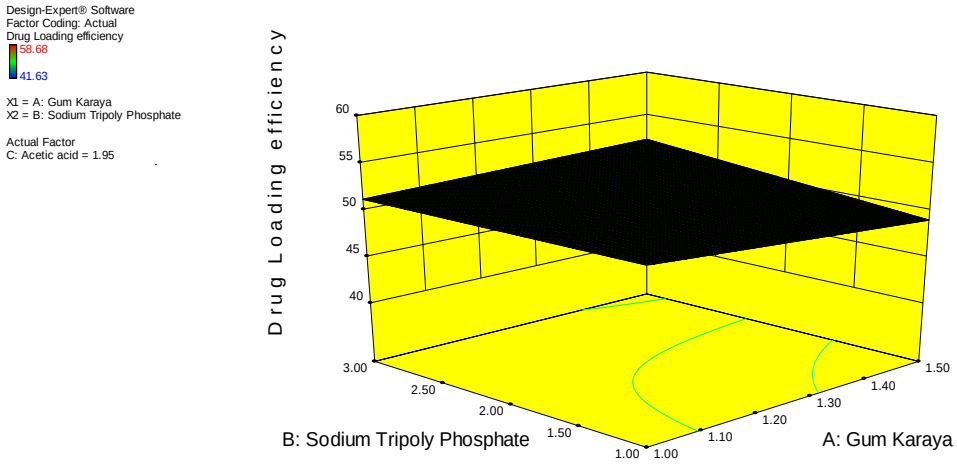
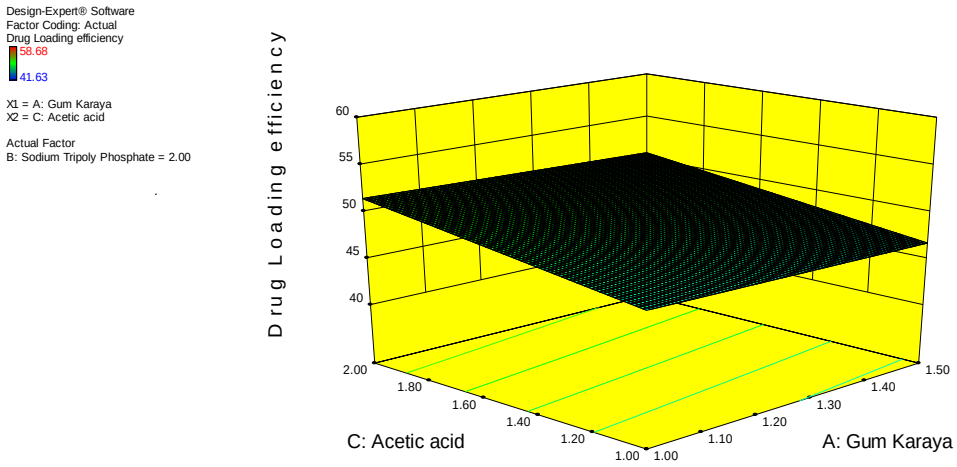


Figure 2: Cube plot showing the effect of polymers concentration on efficiency of drug loading


Design-Expert® Software
Factor Coding: Actual
Drug Loading efficiency
58.68
41.63
X1 = A: Gum Karaya
X2 = B: Sodium Tripoly Phosphate
Actual Factor
C: Acetic acid = 1.95
Drug Loading efficiency
60
55
50
45
40
3.00
2.50
2.00
1.50
1.00
B: Sodium Tripoly Phosphate
1.00
1.10
1.20
1.30
1.40
1.50
A: Gum Karaya


Design-Expert® Software
Factor Coding: Actual
Drug Loading efficiency
58.68
41.63
X1 = A: Gum Karaya
X2 = C: Acetic acid
Actual Factor
B: Sodium Tripoly Phosphate = 2.00
Drug Loading efficiency
60
55
50
45
40
2.00
1.80
1.60
1.40
1.20
1.00
C: Acetic acid
1.00
1.10
1.20
1.30
1.40
1.50
A: Gum Karaya

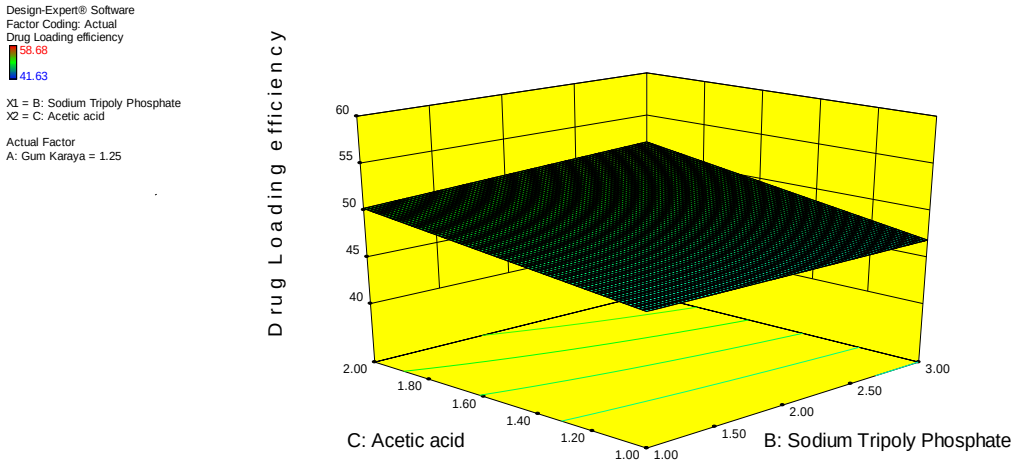

Design-Expert® Software
Factor Coding: Actual
Drug Loading efficiency
58.68
41.63
X1 = B: Sodium Tripoly Phosphate
X2 = C: Acetic acid
Actual Factor
A: Gum Karaya = 1.25
Drug Loading efficiency
60
55
50
45
40
2.00
1.80
1.60
1.40
1.20
1.00
C: Acetic acid
1.00
1.50
2.00
2.50
3.00
B: Sodium Tripoly Phosphate

Figure3. 3D surface plot showing the effect of polymer concentration on efficiency of drug loading

Determination of Drug Loading and Encapsulation Efficiency

All batches show percent entrapment more than 50% and it is found that entrapment of drug increases withan increase in the amount of the polymer. Formulation CFM3 shows maximum entrapment whereas formulationCFM3 shows minimum entrapment of the cefdinir in the polymer as shown in table 4.

Table4.Determination of Drug Loading and Encapsulation Efficiency

S.No	Parameters	Formulation					
		CFM1	CFM2	CFM3	CFM4	CFM5	CFM6
1	Drug loading (%) *	40.52	41.22±0.21	41.26±0.33	33.69±0.25	33.98±.17	34.52±0.12
2	Encapsulation efficiency (%) *	61.06±0.21	61.84±0.25	61.90±0.58	50.41±0.54	50.89±0.36	51.24±0.74

* Mean±S.D

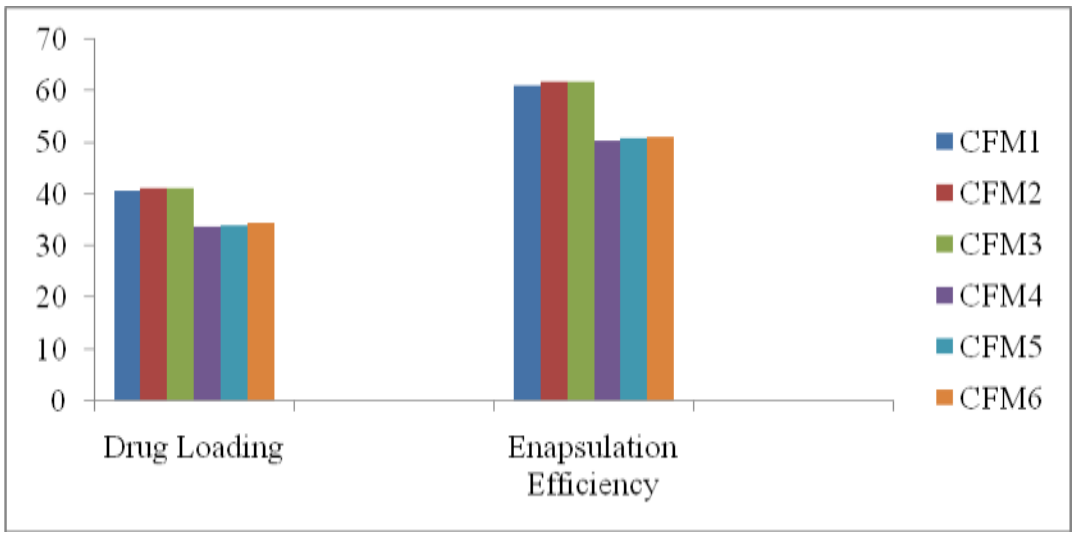


Figure 4. Comparative % of Drug loading and Encapsulation Efficiency Analysis

Determination of *In-vitro* Buoyancy of Microspheres

The formulated batches of floating microspheres of cefdinir showed average buoyancy more than 90%.Amongst the batches of prepared microspheres, batch CFM3 showed highest buoyancy (100%). Microspheres as shown in table 5.

Table 5.Determination of *In-vitro* Buoyancy of Microspheres

S.No	Formulation Code	Percentage %of *In-vitro* Buoyancy of Microspheres
1	CFM1	90.25 %
2	CFM2	95.87%
3	CFM3	100%
4	CFM4	100%

5	CFM5	100%
6	CFM6	100%

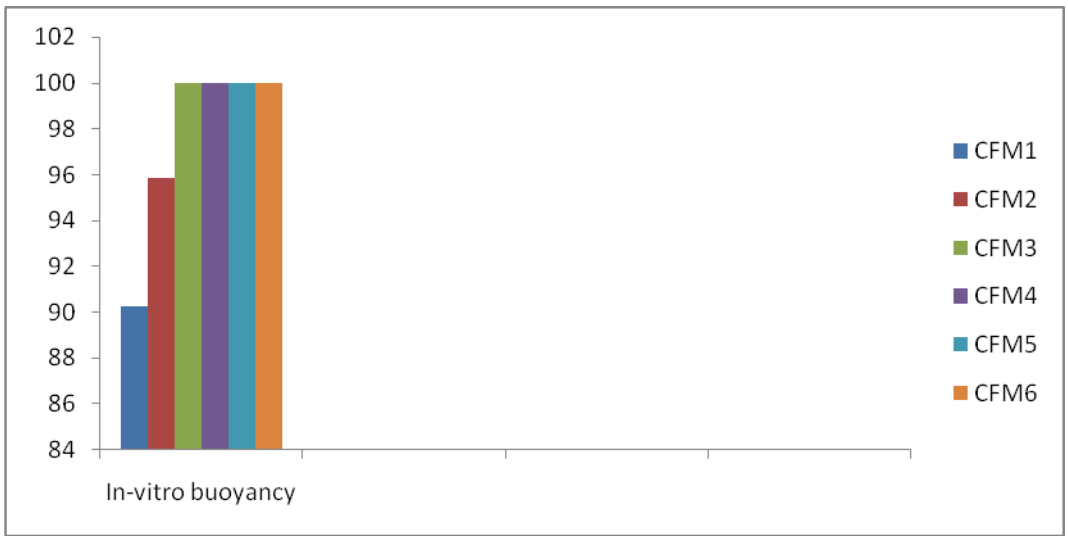


Figure5 . % of *In vitro* Buoyancy Analysis

Table 6.Equilibrium Swelling Studies

S.No	Formulation Code	Water *	S.G.F (pH1.2) *	S.G.F (pH7.4) *
1	CFM1	0.61±0.10	0.76±0.14	0.89±0.11
2	CFM2	0.73±0.11	0.79±0.24	0.89±0.09
3	CFM3	0.74±0.15	0.80±0.09	0.90±0.13
4	CFM4	0.40±0.13	0.64±0.14	0.78±0.06

5	CFM5	0.39±0.09	0.66±0.21	0.78±0.14
6	CFM6	0.37±0.04	0.68±0.23	0.79±0.25

*Mean±S.D

From the Drug Loading, Encapsulation Efficiency, Swelling Studies and *In-vitro* Buoyancy Studies the CFM1, CFM2, AND CFM3 were optimized. These three formulations were subjected to *In-vitro* release studies

Comparative *In-vitro* Cumulative (%) Percentage of Drug Release.

Cefdinir release was higher in the case of microspheres preparedat a higher agitation speed but the difference in drug release was not statisticallysignificant . No significant effect of solvent composition was observed on the *invitro* release of Cefdinir results are showed as Table .7 and Fig 6.

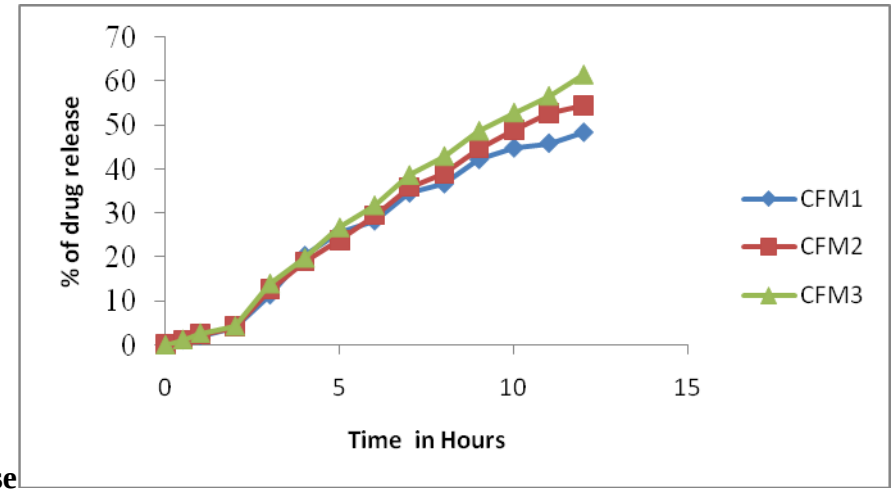
Table 7 Comparative *In-vitro* Cumulative (%) Percentage of Drug Release.

S.No	Time hrs	Cumulative Percentage (%) of Drug Release *		
		CFM1	CFM2	CFM3
1	0	0	0	0
2	0.5	1.12±0.12	1.13±0.94	1.19±0.38
3	1	2.10±0.09	2.32±0.62	2.65±1.03
4	2	4.23±0.03	4.12±0.47	4.25±0.94

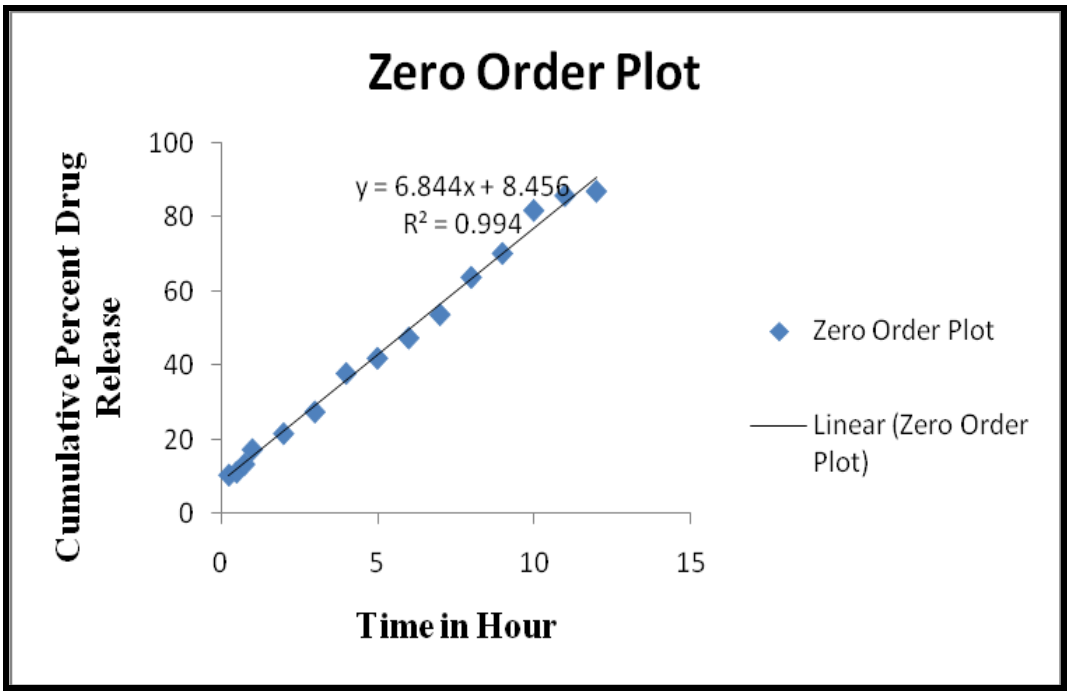
5	3	11.38±0.34	12.65±0.38	13.93±0.72
6	4	20.35±0.73	18.85±0.36	19.75±0.93
7	5	25.63±0.46	23.75±0.27	26.69±0.74
8	6	28.25±0.72	29.45±0.36	31.74±1.82
9	7	34.63±0.93	35.86±0.98	38.63±0.73
10	8	36.74±0.37	38.97±1.02	42.94±0.92
11	9	42.28±0.95	44.69±0.27	48.69±0.45
12	10	44.93±0.28	48.83±0.46	52.83±0.82
13	11	45.99±0.38	52.63±0.28	56.63±0.48
14	12	48.48±0.82	54.48±0.37	61.48±0.73

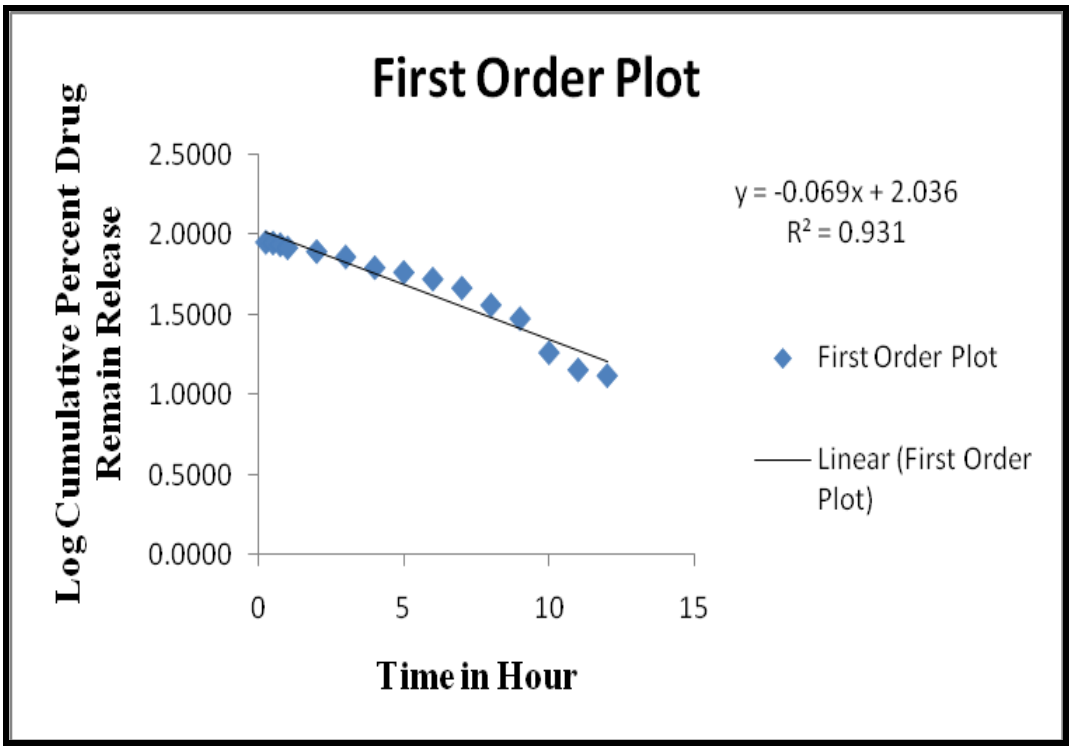
* Mean±S.D; n=3

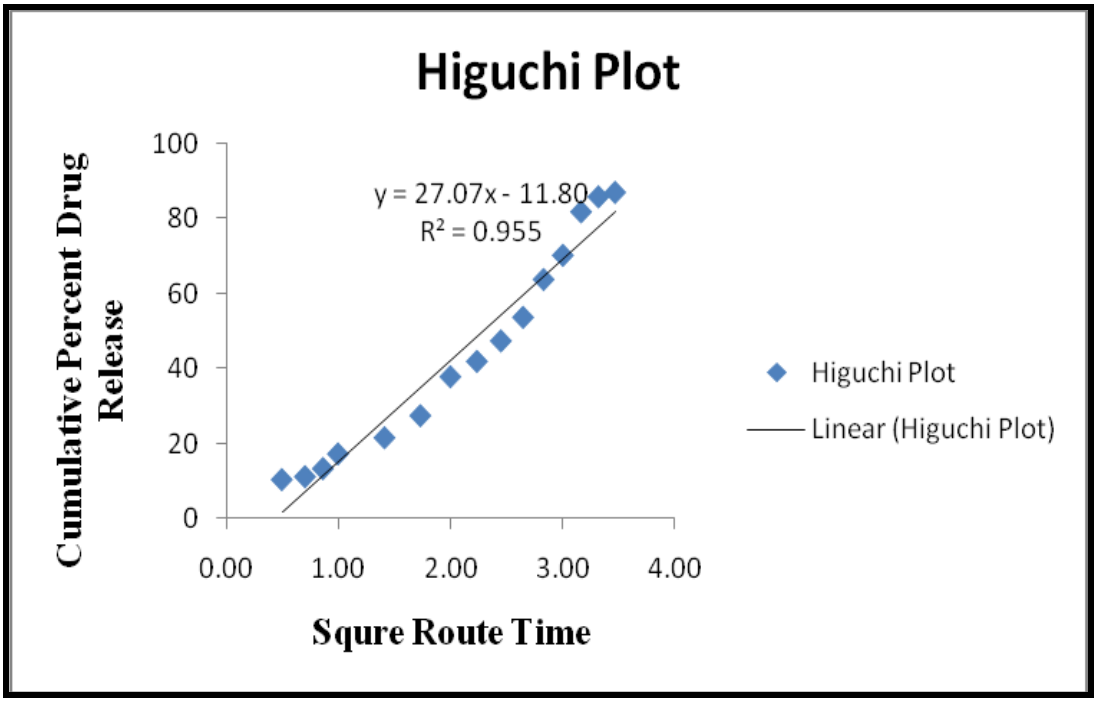
Fig 6.Comparative *In-vitro* cumulative percentage of drug release



Release kinetics for formulation CFM3 fig-7




First Order Plot
Log Cumulative Percent Drug Remain Release
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
0
5
10
15
Time in Hour
y = -0.069x + 2.036
R² = 0.931
First Order Plot
Linear (First Order Plot)


Higuchi Plot
Cumulative Percent Drug Release
100
80
60
40
20
0
0.00
1.00
2.00
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4.00
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Higuchi Plot
Linear (Higuchi Plot)

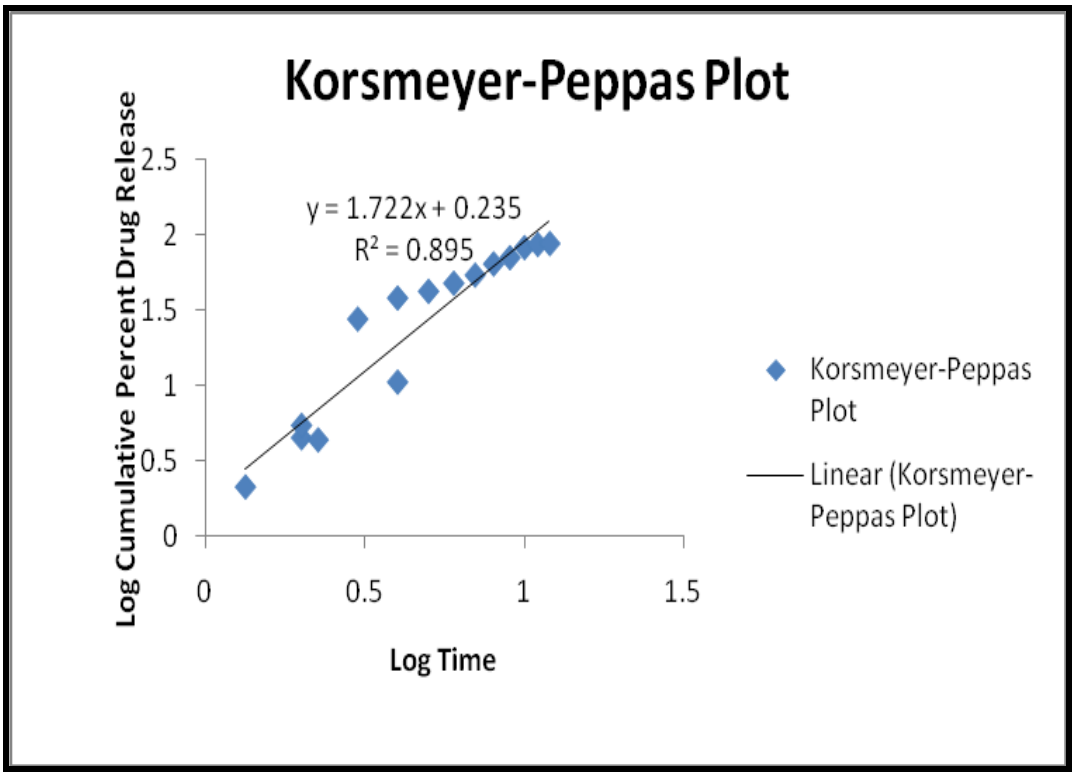


Table 8: Results of curve fitting of the in-vitro release data from different formulation of microspheres in simulated gastric fluid

Formulation Code	Correlation coefficient (r^2)				'n'- Release Exponent
	Zero order [r]	First order [r]	Higuchi [r]	Korsmeyer-Peppas [r]	
CFM1	0.929	0.912	0.933	0.836	0.923
CFM2	0.956	0.908	0.911	0.838	0.903

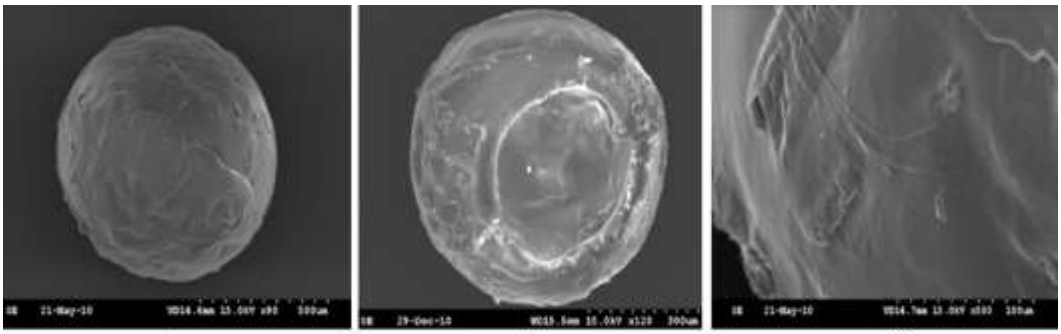
CFM3	0.994	0.931	0.955	0.895	0.835

n=Diffusion exponent related to mechanism of drug release, According to equation $Mt/M\infty=K\ t^{n}$, r= Correlation coefficient

Form the *In-vitro* drug release the CFM3 shows better release and it's follows non-Fickian drug release so this was subjected to scanning electron microscopy (SEM)

Scanning Electron Microscopy

Graphs show regular shaped microparticles (2.4- 2.95 mm in diameter) having an apparently homogenous and smooth surface with few wrinkles and inward dents due to the collapse of the microcapsule wall during the in situ drying process



A B C

A- Fig 8.General appearance; B- Hollow structure; C- Surface morphology

Stability studies as per ICH

The formulation CFM3 was selected and the stability studies were carried out at accelerated condition of 40±2°C, 75±5% RH conditions, stored in desiccators, the microspheres were packed in muslin cloth which is covered by aluminium foil and kept for period of three months. The microspheres were analyzed periodically for their morphology, swelling ratio, water up take, drug content and *in-vitro* drug release. Results were analyzed by One-way

ANOVA followed by Tukey's test. Differences were considered statistically significant at $p<0.05$.

Conclusion

The Cefdinir floating microspheres were prepared by Capillary Extrusion method is well suited for the successive formulations. The polymers and solvent were chosen has showed more percentage yield of microspheres. The physicochemical properties were characterized by Swelling index, percentage yield, Drug content and Drug entrapment efficiency. The *in-vitro* drug release studies were performed all the formulations shows the controlled release pattern of drug up to 11 h. Surface morphological studies by SEM analysis obtained showed good spherical shape and also surface morphological characters. The satisfactory results were obtained in all prepared formulations and based on the results CFM3 was best one when compared to other. Good correlation was observed between *in-vitro* profiles, revealed the ability of the formulation to reproduce the *in-vitro* release pattern. Hence Cefdinir floating microspheres could be promising one as they, increase bioavailability, minimize the dose, reduces the side effects and improve patient compliance and also Cefdinir might be a right and suitable candidate for oral floating drug delivery via microspheres.

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